Mechanisms of Action of Cancer Chemotherapeutic Agents: DNA-Interactive Alkylating Agents and Antitumour Platinum-Based Drugs

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INTRODUCTION

The long-term understanding of cancer growth is that it is a net result of uncontrolled multiplication of cells that out paces the rate of natural cell death within the tumour mass. The ultimate aim of the tumour is survival by overcoming the many barriers in its path. The imperative need for continual supply of nutrients to new growth areas, for instance, is met through sustained angiogenesis. Another major factor for growth and survival is limitless replicative potential, requiring continued biosynthesis of the genetic material to provide a complementary set of chromosomes in each of the daughter cells following cell division. Inhibiting DNA replication, therefore, affords a logical approach for retarding tumour growth. For this reason, DNA has become a critical target in cancer chemotherapy. Indeed, many of the antitumour agents currently in the cancer armamentarium are DNA-interactive. Among them, the DNA alkylators or cross-linkers, which includes the platinum-based drugs, are the most active available for effective cancer management.

Historically, nitrogen mustard was introduced in 1942 as the first alkylating agent to have clinical utility (Gilman and Phillips, 1946). It was an analogue of the highly toxic sulphur mustard gas, which had been used as a weapon in 1917 during the First World War and later as a therapeutic agent against squamous cell carcinoma (Adair and Bagg, 1931). The advent of nitrogen mustard was the beginning of modern cancer chemotherapy, and it spawned a series of more effective and less toxic alkylating agents that are still in use today. Five major structural classes of alkylating agents are of considerable interest: the nitrogen mustards, the aziridines, alkyl sulphonates, the nitrosoureas and the mechanistically distinct platinum-containing drugs.

GENERAL MECHANISM OF ACTION

By virtue of their high chemical reactivity, either intrinsic or acquired in a biological environment, all alkylating agents form covalent linkages with macromolecules having nucleophilic centres. They have no specificity, but the chance reaction with DNA forms the basis for the antitumour effects. Bifunctional alkylating agents form covalent bonds at two nucleophilic sites on different DNA bases to induce interstrand (between two opposite strands) and/or intrastrand (on same strand) cross-links. Such cross-links can have either a 1,2 or 1,3 configuration (Figure 1). Monofunctional agents have only one alkylating group and, therefore, cannot form crosslinks. The traditional alkylators interact with DNA (usually the N7 position of guanine) through an alkyl group, and this is distinct from a platinum-containing drug, which, although loosely referred to as an alkylating agent, forms covalent links between adenine and/or guanine bases via the platinum atom. Irrespective of the specific mechanisms involved in the formation of adducts, the end effect of these DNA-interactive agents is to inhibit DNA replication, which in turn may affect the production of RNA and protein (Lawley and Brookes, 1965). These reactions, unfortunately, do not discriminate between normal and tumour DNA, which is a characteristic of all antitumour agents that leads to side effects and the associated low therapeutic indices. Any

*The Cancer Handbook 1*st *Edition*. Edited by Malcolm R. Alison

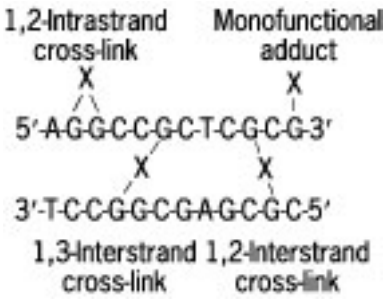

Figure 1 Monofunctional adducts and 1,2- and 1,3-interstrand and intrastrand cross-links induced by DNA interactive agents. X = antitumour agent.

antitumour selectivity that is observed is dependent on the extent of covalent interactions induced by the drug that affects distortions and unwinding in DNA. Such changes in the superhelical structure are then processed as distinct signals that determine whether a cell lives or dies. When DNA is damaged, these signals inhibit cell cycle progression, which is a process that the cell activates to allow DNA repair to proceed and, thereby, prevent replication of new DNA on a damaged template or prevent damaged chromosomes to be passed on to daughter cells. Thus, as a rule, drugs that interact with DNA affect the cell cycle, and whether a cell survives or dies depends on the extent of interaction between the drug and DNA, and how rapidly the adverse effects of that interaction can be neutralized through DNA repair. Indeed, one of the mechanisms of resistance of tumour cells to alkylating and platinum agents is attributed to enhanced repair of cross-links.

In this chapter, the mechanism of action of some established DNA interactive agents will be discussed, but for many the detailed information is scant and very little is known regarding events following DNA damage. For this reason, emphasis will be placed on cisplatin, which has been studied in greater detail and, therefore, allows us to appreciate the complexity of the molecular pathway from DNA damage to cell death. Similar or overlapping pathways probably exist for the other DNA-interactive drugs.

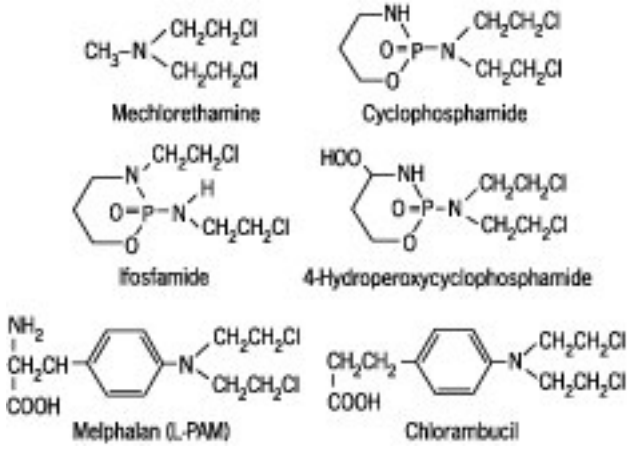

Figure 2 Structures of selected members of the nitrogen mustard family of drugs.

NITROGEN MUSTARDS

Since the mid-1940s, hundreds of nitrogen mustard-based alkylating agents have been evaluated for their potential as antitumour agents. However, only a handful have found a place in medical oncology as therapeutic agents. These include nitrogen mustard (mechlorethamine), chlorambucil, melphalan, cyclophosphamide and its activated prodrug form 4-hydroperoxycyclophosphamide and ifosfamide (Figure 2). The common structural feature is the bischloroethyl group, which is the precursor for the activated function that predominantly alkylates the N7 of guanine, although minor alkylation reactions can also occur at other sites, including the O6 position of guanine, and N3 and N7 of adenine (Colvin *et al*., 1999). Mechlorethamine reacts with guanine following spontaneous activation at physiological pH. The rapid rate of activation of this agent, however, is the major cause of side effects. For this reason, other members of the nitrogen mustard family are of greater interest as they have been structurally modified to regulate the generation of the active species. Cyclophosphamide, for instance, is highly stable and requires the hepatic mixed function oxidase system to activate the molecule metabolically (Sladek, 1987). Although the metabolism of cyclophosphamide is complex, the product 4-hydroxycyclophosphamide is considered the most significant. This metabolite distributes throughout the body, including the tumour where spontaneous degradation occurs to form phosphoramide mustard or nornitrogen mustard (Figure 3). It is useful to note that a byproduct of cyclophosphamide activation is acrolein, which is responsible for haemorrhagic cystitis as a serious side effect (Cox, 1979).

The chloroethyl group of the biotransformed mustard is very important in the reaction that ensues with macromolecules. Before its reaction, however, the group cyclizes to the imonium (aziridinium) ion, which is the highly reactive alkylating moiety that interacts with the DNA molecule (Figure 4). However, it is uncertain whether alkylation by the imonium occurs directly or via rearrangement to the reactive carbonium ion intermediate (Colvin *et al*., 1999). Since the two chloroethyl groups in the nitrogen mustard drugs are retained in phosphoramide and nornitrogen molecules, a bifunctional reaction with macromolecules ensues. Thus, each drug molecule forms adducts with two individual nucleotide bases through a sequential alkylation process; that is, a monofunctional adduct is formed first and this is followed by the second adduct in the opposite strand of the DNA. This bifunctional reaction, thereby, generates an interstrand cross-link between the two strands of DNA in the helix. Both 1,2- and 1,3-cross-links are feasible from an energetic consideration, but it appears that the 1,3-cross-link is favoured by the mustards (Colvin *et al*., 1999). The interstrand cross-link is considered critical in preventing the two opposing strands from separating during replication, which leads to inhibition of DNA synthesis. Cross-links can also occur on the same strand of DNA and

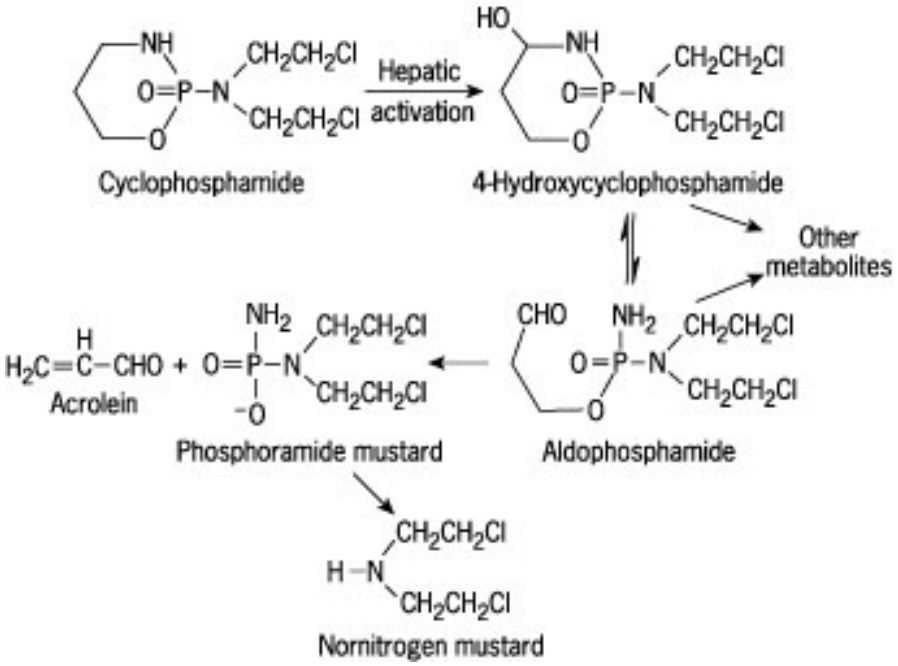

Figure 3 Metabolism of cyclophosphamide to reactive products. (Adapted from Sladek, 1987 and Pratt *et al*., 1994).

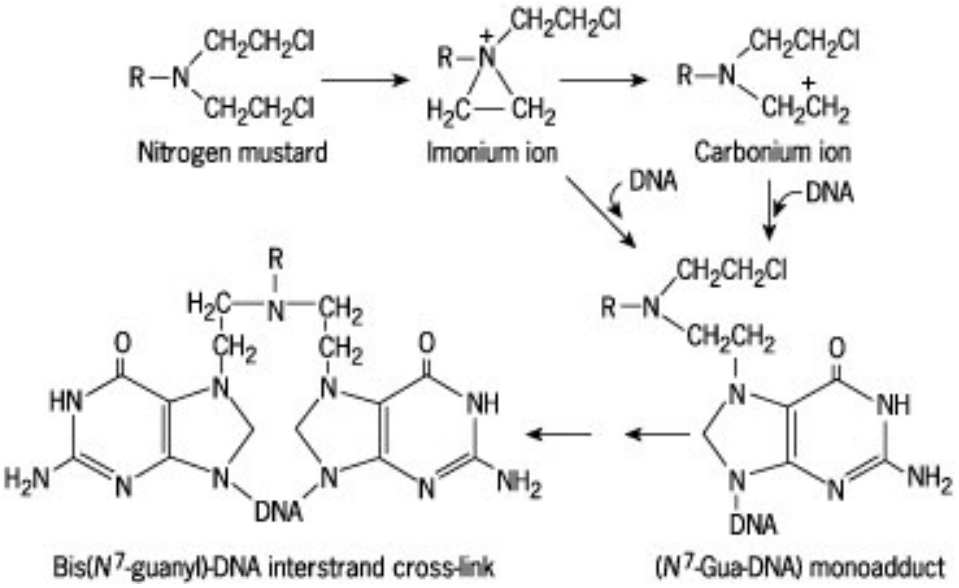

Figure 4 Mechanism of DNA alkylation by a reactive nitrogen mustard molecule and formation of interstrand cross-link. (Adapted from Sladek, 1987 and Pratt *et al*., 1994.)

between DNA and protein, but for mustards these lesions are not considered to be cytotoxic (Colvin *et al*., 1999). Similarly, if the second alkylating reaction is with glutathione, the resulting monofunctional lesion in the DNA also has reduced cytotoxicity. From these considerations, it is reasonable to conclude that the interstrand DNA cross-link is essential for maximal cell killing (Garcia *et al*., 1988). Indeed, bifunctional interstrand adducts are as much as 100-fold more cytotoxic than monofunctional adducts (Roberts *et al*., 1968).

Like cyclophosphamide, the closely related ifosfamide also requires metabolic activation along an identical pathway (Sladek, 1987), but the structural variation in the analogue is such that the rate of hepatic activation is reduced, which, thereby, decreases drug potency. For this reason, about four times as much drug is required to give the same cytotoxic effects as cyclophosphamide (Colvin, 1982). However, the cumulative amount of acrolein produced is substantially greater, which becomes a dose-limiting factor that requires the clinical use of the thiol-containing agent Mesna to inactivate the toxic product. The presence of

the electron-withdrawing aromatic ring in melphalan and chlorambucil also reduces the rate of formation of the imonium ion. As a result, the potency of these molecules is also reduced. Metabolic activation appears not to be necessary for these specific nitrogen mustards. However, melphalan is actively transported in certain tumour cells by a high-affinity carrier system that can increase the activity of the molecule (Vistica, 1979). An alternative application of nitrogen mustards in purging leukaemic cells from bone marrow aspirates has required the design of the prodrug 4-hydroperoxycyclophosphamide. This drug does not require metabolic activation for activity, and is, therefore, very effective in an *ex vivo* setting (Yeager *et al*., 1986).

AZIRIDINES

The aziridines, also known as ethylenimines, are a family of alkylating agents that contain three-membered aziridine rings. Members of this family include triethylenemelamine, triethylenethiophosphoramide (thio-tepa), and mitomycin C (Figure 5). Hexamethylmelamine (Altretamine), which is a close relative of triethylenemelamine, is also a family member, although the classical aziridine ring is absent. The aziridine ring is structurally similar to that present in the reactive imonium ion formed by nitrogen mustards. However, since the aziridine ring does not carry a charge, these drugs are much less reactive than the mustards.

The aziridines are activated spontaneously or by an enzymatic oxidative reaction. Following activation, alkylation can occur at a number of nucleophilic sites in DNA, RNA, protein and other molecules such as glutathione. With DNA, alkylation reactions of thio-tepa have been reported at a number of sites, including N1 of thymine, O2 of cytosine, N1, N6 and N7 of adenine, and N1, N7 and O6 of guanine (Maanen *et al*., 2000). However, the preferential target is the N7 position of guanine, with subsequent formation of guanine–guanine (GG) and adenine–guanine (AG) 1,2-interstrand cross-links (Andrievesky *et al*., 1991). Two possible pathways for the formation of DNA adducts with aziridines are exemplified with thio-tepa (Figure 6). One pathway (pathway A in Figure 6) involves a sequential reaction that results in cross-link formation. In the second reaction pathway (pathway B), resolved using radiolabelled drug in L1210 leukaemic cells (Egorin and Snyder, 1990; Musser *et al*., 1992), hydrolytic cleavage liberates the aziridine groups, which induce monofunctional adducts that subsequently lead to DNA strand breaks and cell death. In this respect, thio-tepa functions as a prodrug for the alkylating aziridine molecule (Maanen *et al*., 2000).

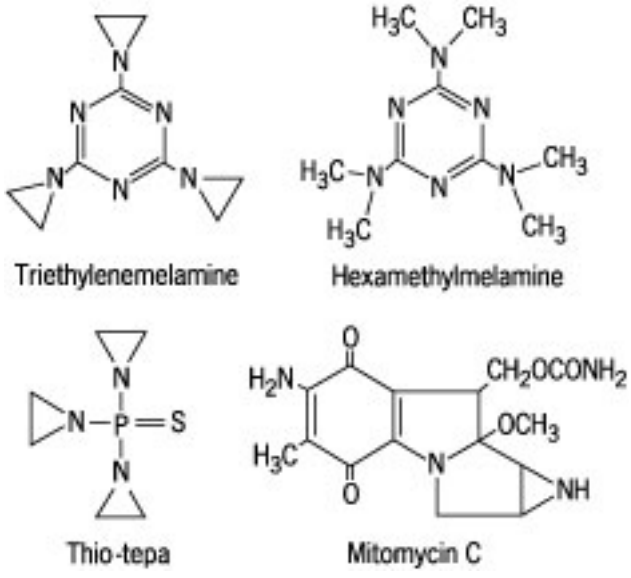

Figure 5 Structures of aziridines and hexamethylmelamine.

Triethylenemelamine probably undergoes reactions with DNA that are similar to thio-tepa. Mitomycin C, on the other hand, requires an enzymatic reduction to activate the aziridine ring before reaction can occur with DNA initially to form a monofunctional adduct (D'Incalci *et al*., 1992; Pratt *et al*., 1994). The preferential alkylation site for this initial reaction appears to be the N2 position of guanine. A second alkylation reaction with the opposite DNA strand follows the spontaneous intramolecular elimination of the carbamate group and results in interstrand cross-links between guanine bases (Figure 7). However, alkylation is preferred in 5′C–G3′ sequences to give the 1,2-GG cross-links in DNA. Metabolic activation also plays an important role in activating hexamethylmelamine. Hepatic mixed function oxidases sequentially metabolize the methyl groups in the molecule to alcohol derivatives, which rearrange to reactive iminium ions that then alkylate guanine bases. In Figure 8, the conversion of hexamethylmelamine to pentamethylmelamine is demonstrated, but subsequent metabolism of this product can lead to the loss of all six methyl groups and potential generation of an iminium ion from each methyl group metabolized. Hexamethylmelamine derivatives, such as trimelamol, have also been designed that do not appear to require metabolic activation for generation of the reactive iminium ion (Siddik and Newman, 1994).

ALKYL SULPHONATES

Busulphan is the best known of the alkyl sulphonates, and has a linear symmetrical chemical structure that facilitates cross-link formation. However, the mechanism of alkylation of this molecule is different. Unlike the mustards and the aziridines, which must first generate reactive species, busulphan interacts directly with the N7 position of guanine and leads to the formation of DNA mono- and then bi-adducts, with release of methyl sulfonate groups (Figure 9). Interstrand cross-links between guanines have been demonstrated for busulphan (Tong and Ludlum, 1980) and, as with the mustards, this is considered the cytotoxic lesion (Bedford and Fox, 1983).

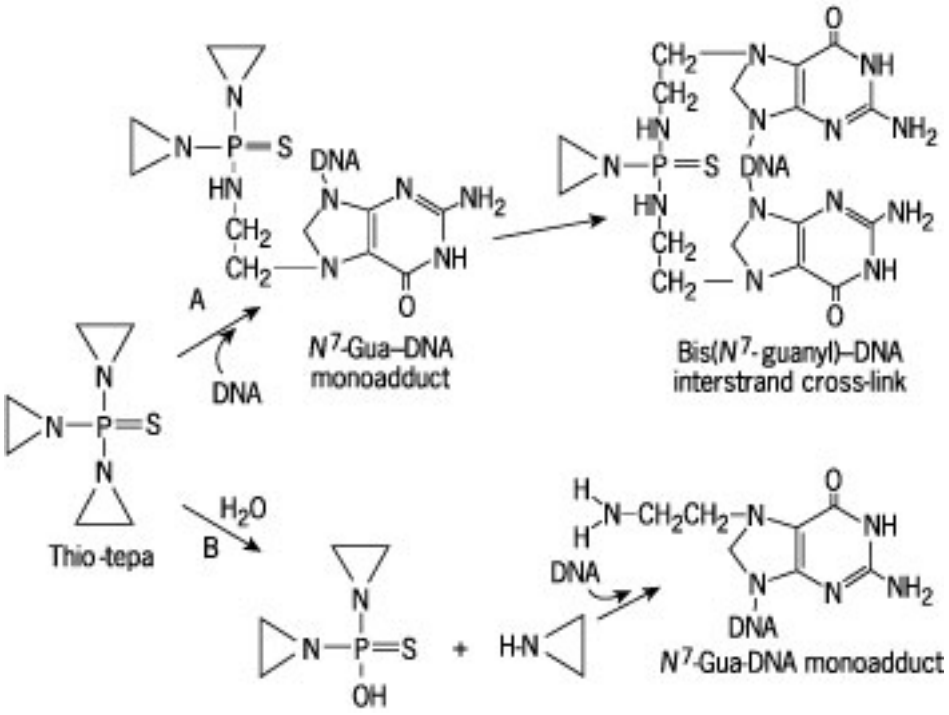

Figure 6 Mechanisms involved in the formation of DNA interstrand cross-links (pathway A) and monofunctional adducts (pathway B) by thio-tepa. (Adapted from Maanen *et al*., 2000.)

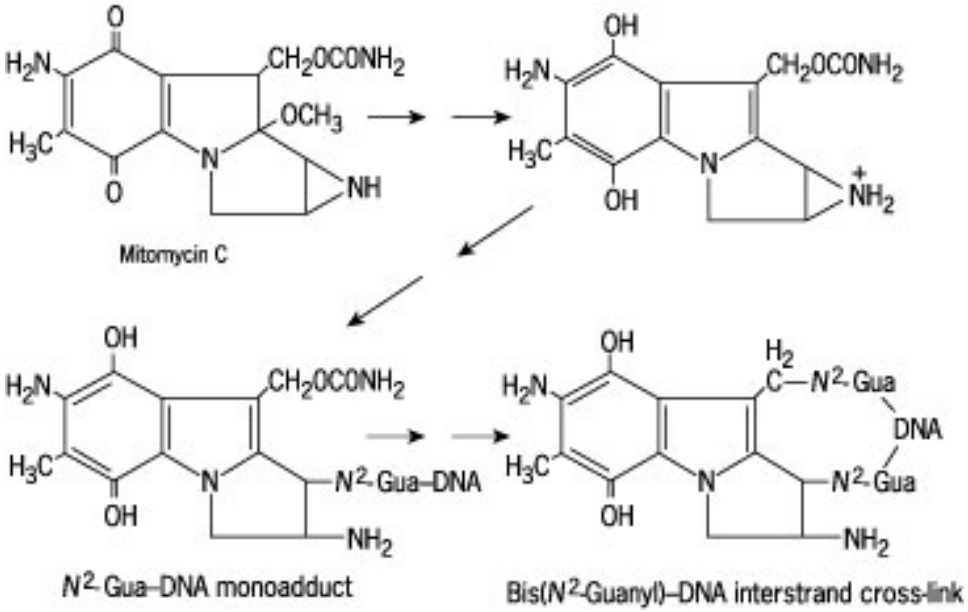

Figure 7 Reaction of mitomycin C with DNA to form cross-links between guanine bases. (Adapted from Pratt *et al*., 1994.)

NITROSOUREAS

Much of the early focus on nitrosoureas as antitumour agents came from studies of Montgomery and co-workers at the Southern Research Institute in Birmingham, AL, USA (Reed, 1987). The extensive structure–activity studies over many years established the foundation that eventually led to the discovery of the more useful 2-chloroethylnitrosoureas (CENUs) that are currently in clinical use. A number of nitrosoureas are of clinical interest, and include BCNU (carmustine), CCNU (lomustine), methyl-CCNU (semustine) and chlorozotocin (Figure 10).

In general, the CENUs are highly unstable and rapidly undergo spontaneous transformation to yield a number of products (Figure 11). A most significant product, however, is the highly unstable 2-chloroethyldiazene hydroxide,

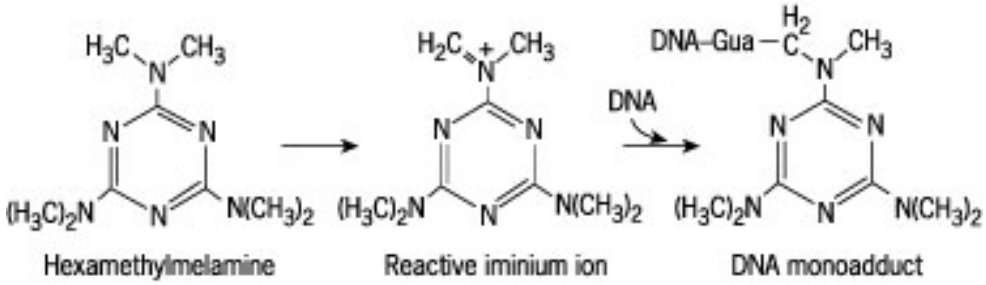

Figure 8 Conversion of HMM to an iminium ion and the formation of DNA adduct. (Adapted from Pratt *et al*., 1994.)

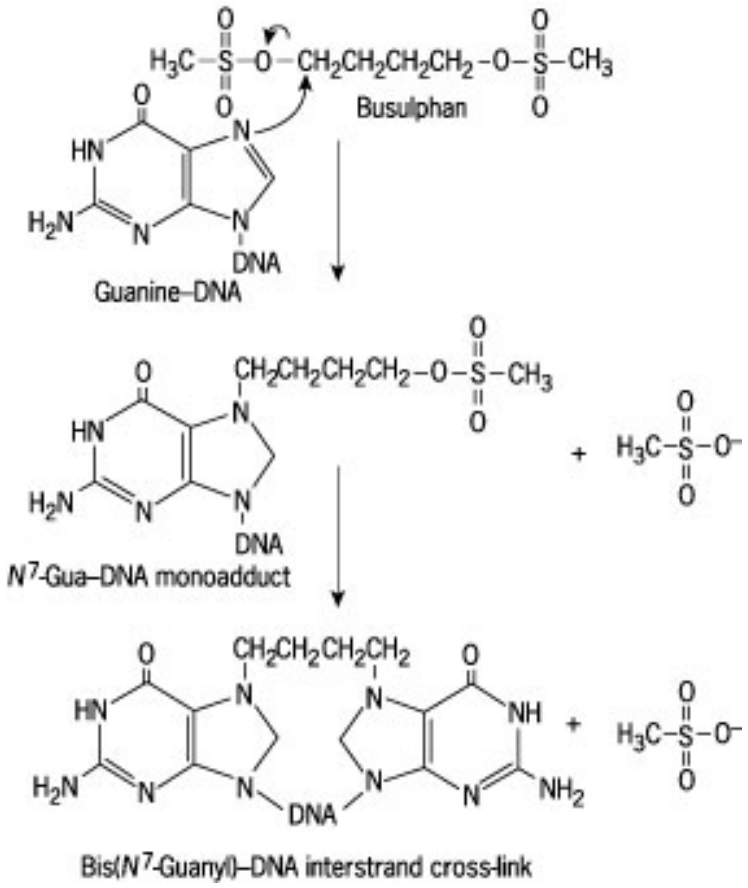

Figure 9 Mechanism of formation of DNA interstrand cross-links induced by busulphan.

which transforms to the alkylating 2-chloroethylcarbonium ion (Ludlum, 1997). Although this reactive ion can alkylate nucleophilic sites in DNA to yield a number of modified DNA bases, the N7 position of guanine appears to be a predominant site for alkylation, particularly when this base is in the middle of a run of three or more guanines in DNA (Reed, 1987; Lemoine *et al*., 1991). In contrast to other DNA-reactive agents, the CENUs also alkylate the O6 site of guanine to a large extent. The significance of O6 alkylation can be recognized from the knowledge that cytotoxicity correlates inversely with cellular activity of the DNA repair enzyme O^6-alkylguanine–DNA alkyltransferase, which removes the monofunctional O6 adduct from the DNA (Pratt *et al*., 1994). Thus, when the O^6-alkyltransferase is overexpressed, sensitivity of tumour cells to CENUs diminishes. The DNA monoadducts are chloroethyl derivatives, but substantial amounts of hydroxyethyl adducts are also formed (Figure 11). It is possible that hydroxyethyl adducts could arise from hydrolysis of chloroethylated bases, but it appears more likely that other reactive intermediates of CENUs are involved in the transfer of hydroxyethyl groups to DNA. One likely explanation is that the CENUs can cyclize (Figure 11), and during decyclization the chloride group is replaced by the hydroxyl group, which can then form the alkylating 2-hydroxyethyl carbonium ion (Eisenbrand *et al*., 1986).

The initial monofunctional adduct formed by the CENU is converted to an alkyltransferase-resistant 1,2-cross-link

through labilization of the alkylating chloroethyl group on the initial site and reaction with a nucleophilic site on a second DNA base (Figure 12, pathway A). This explains why CENUs, such as CCNU, with only a single chloroethyl side chain have the capacity to cross-link DNA. Reaction kinetics indicate that the initial alkylation to form the DNA monoadduct occurs very rapidly (usually within minutes), whereas the conversion to the cross-link can take 6–12 h. Chemical structures of two DNA lesions have been identified as guanine–guanine (through N7 positions) and N^3-cytosine-N^1-guanine (CG) 1,2-cross-links. Although the chemical reaction leading to the bisguanine (GG) cross-link at the N7 positions is consistent with the characterized N^7-guanine monofunctional adduct, the CG cross-link through the N1 position of guanine is not as straightforward to comprehend, particularly since alkylation at the N1 site is rare. It is most likely that the CG crosslink occurs through an initial O^6-guanine adduct, which cyclizes to the O^6-ethanoguanine intermediate that then reacts with cytosine (Figure 12, pathway B). Steric considerations suggest that the CG cross-link is interstrand and the GG cross-link is intrastrand (Ludlum, 1997). Irrespective of their nature, both GG and CG cross-links correlate strongly with cytotoxicity.

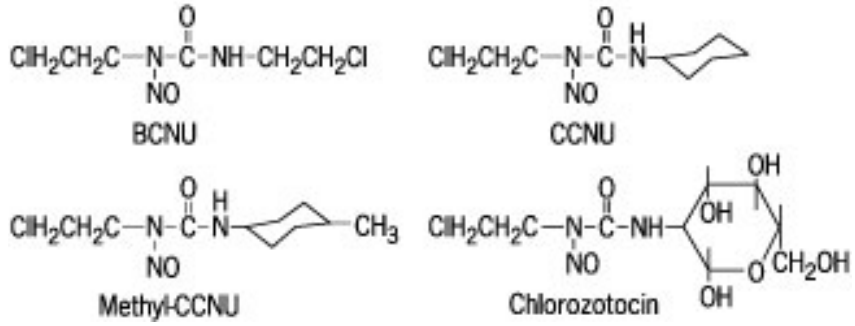

Figure 10 Structures of selected 2-choloroethylnitrosourea antitumour agents.

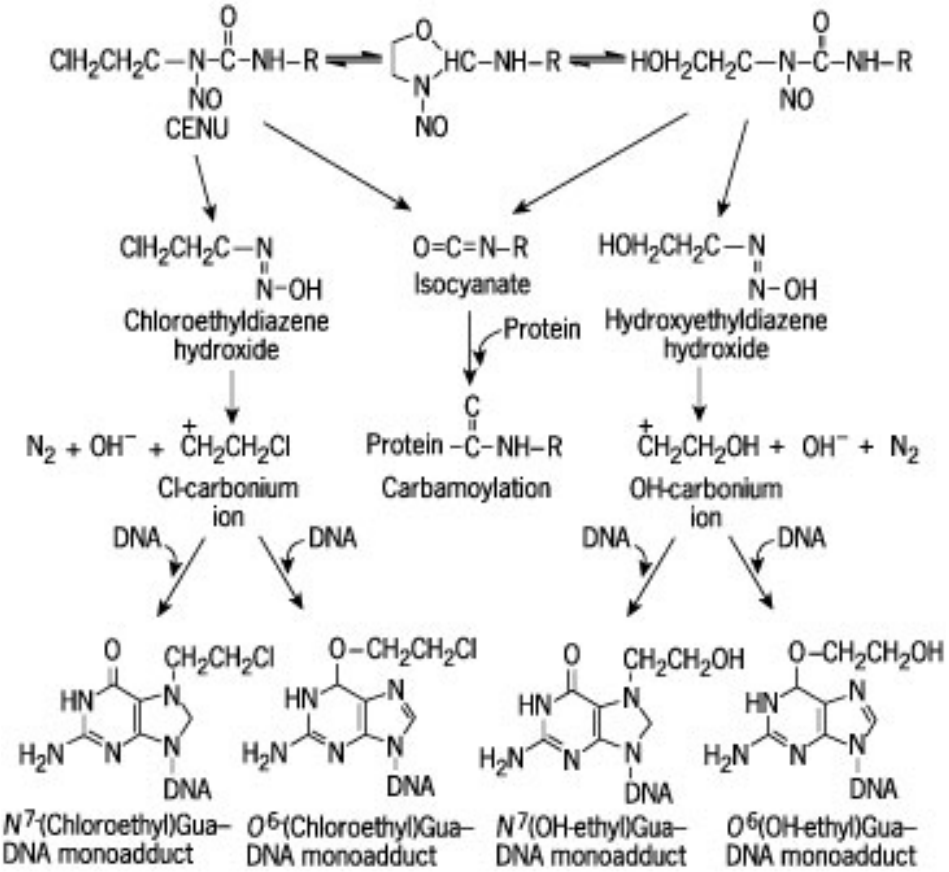

Figure 11 General reaction of a 2-chloroethylnitrosourea (CENU) with DNA to form monoadducts as the initial step in cross-link formation. (Adapted from Eisenbrand *et al*., 1986, Pratt *et al*., 1994 and Ludlum, 1997.)

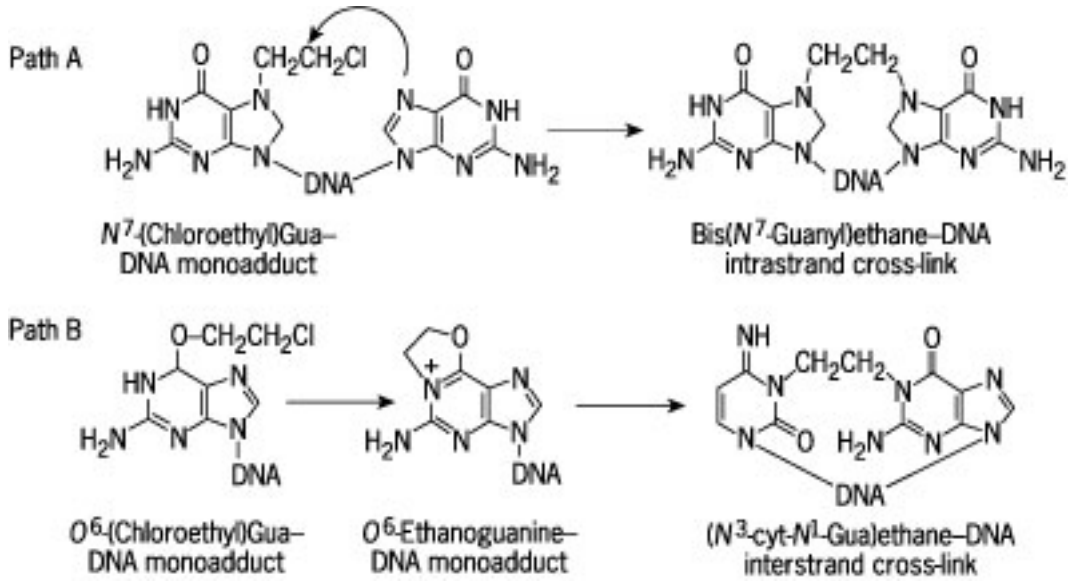

Figure 12 Mechanisms responsible for the conversion of monofunctional adducts of CENU to GG intrastrand (pathway A) and CG interstrand (pathway B) cross-links. (Adapted from Ludlum, 1997.)

A second interesting product of spontaneous CENU transformation is the isocyanate species (Figure 11), which is formed in varying amounts depending on the chemical structure of the CENU. Although isocyanates can carbamoylate a range of proteins at the ε-amino group of lysine, including nuclear histone and nonhistone proteins, there is no correlation between carbamoylation activity and cytotoxicity (Lemoine *et al*., 1991). Chlorozotocin, for instance, has low carbamoylating activity, but retains antitumour activity. However, there is disagreement whether carbamoylation reaction contributes to the side effects of CENUs. Although the rate and extent of carbamoylation of protein may not be associated with bone marrow toxicity of some nitrosoureas (Reed, 1987), this does not necessarily preclude other CENUs that possess a distinctly different carbamoylating isocyanate function in the molecule from inducing myelotoxicity (Ali-Osman *et al*., 1985).

PLATINUM-BASED AGENTS

The platinum drug cisplatin (*cis*-diamminedichloroplatinum(II)) is perhaps one of the most effective antitumour agents currently in clinical use. Although the drug had previously been known as Peyrone's salt for over 100 years, it was not until 1969 that its antitumour effects were first recognized through a serendipitous finding. In an experiment designed to determine how *E. coli* would behave in an electric field, Rosenberg and colleagues (Rosenberg, 1980) passed an electrical current via platinum electrodes through a bacterial culture, which contained nutrients that included ammonium chloride as a source of nitrogen. It was noted that the bacteria stopped dividing, but continued to grow and became filamentous. Subsequent investigations to explain this observation led to the isolation from the culture of several divalent and tetravalent platinum-containing products that formed from the reaction between the electrodes and the culture medium (presumably ammonium chloride). The most effective of the agents was identified as cisplatin, which was subsequently developed as an antitumour agent. The drug became approved for clinical trials in 1972.

There is no question that cisplatin has had a major impact in the treatment of several important cancers, such as those of the ovary, testes and head and neck (Prestayko *et al*., 1979), but its clinical utility can often be compromised by side effects and the onset of tumour drug resistance. It was indeed the dose-limiting nephrotoxicity that led to the search for a less toxic platinum analogue. The eventual identification of the clinically active carboplatin in the early 1980s by Harrap and his colleagues was a result of an intensive laboratory-based effort that required an initial examination of over 300 analogues (Kelland *et al*., 1999). Although carboplatin has been important in overcoming the irreversible renal damage and the peripheral neuropathy associated with cisplatin use in patients, it is, however, fully cross-resistant with the parent molecule (Gore *et al*., 1989; Eisenhauer *et al*., 1990). Therefore, greater attention has been devoted recently to identify analogues capable of circumventing cisplatin resistance, and a few have been introduced into clinical trials with various degrees of success (Kelland *et al*., 1999). The 1,2-diaminocyclohexane (DACH)-based oxaliplatin is fulfilling its potential against specific refractory cancers (Faivre *et al*., 1999), but the underlying basis for its activity is yet to be defined. Indeed, this and other analogues, such as ZD0473 (Kelland *et al*., 1999), are still under active clinical investigations and, regardless of the fact that current investigations are intense, it may be some time before their mechanism of action will become fully appreciated. However, it is useful to note that according to the results of the DISCOVERY computer program analysis by the National Cancer Institute in the USA, cisplatin and

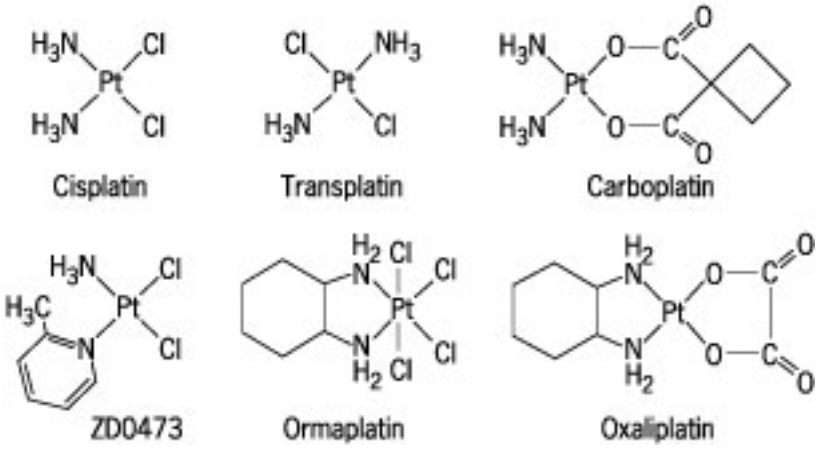

Figure 13 Structures of cisplatin and selected analogues of clinical and mechanistic interests.

its analogues fall into at least 13 clustered regions, each reflecting a distinct mechanism of action (Tanimura *et al*., 1995). These mechanisms are also not known at the present time. Indeed, almost 30 years after its clinical acceptance as a potent antitumour drug, we are still searching for answers to explain exactly how cisplatin works. Therefore, this section will focus primarily on our present understanding of the mechanism of action of cisplatin.

Some of the platinum drugs of interest, either clinically or from the perspective of understanding the mechanism of action, are shown in Figure 13. Cisplatin is a square-planar inorganic molecule, which has the central platinum in a divalent state. Other platinum(II) agents have similar configurations. In contrast, the tetravalent platinum(IV) compounds, such as ormaplatin (tetraplatin), have an octahedral structure, but they are considered as prodrugs for the corresponding active platinum(II) structures. Cisplatin has a rigid structure, with two labile chloro and two stable ammine ligands in a *cis* configuration. This is critical for antitumour activity, as the isomer transplatin, with a *trans* geometry, is relatively ineffective. A few active experimental *trans*-platinum(II) agents, however, transcend the absolute requirement for a *cis* configuration (Perez *et al*., 1999), but the reason for this is unclear. The cytotoxic activity of cisplatin has sparked considerable interest in other metal-based agents, but none of the possible metal alternatives, including gold, ruthenium, rhodium and palladium, provide the optimal chemical environment for active antitumour drugs.

Chemistry of Cisplatin as a Basis for Activity

Cisplatin is considered a very potent antitumour agent, yet from a chemical perspective the molecule itself is inert, and unable to react with biological macromolecules. Like some alkylating agents, the neutral drug molecule needs to be converted to a reactive form. This occurs nonenzymatically in solution, where displacement reactions result in stepwise exchange of the labile chloro ligands with water molecules (el Khateeb *et al*., 1999; Kelland, 2000). Such aquations also occur with other platinum analogues, and lead to several species that exist in equilibrium, as exemplified with cisplatin in Figure 14. The charged aquated species are highly reactive, but the chloro-monoaquo species is the most significant from the perspective of interaction with DNA at physiological pH. The reactive aquated species, however, can also be inactivated through nonspecific interaction with many endogenous nucleophilic molecules and macromolecules, such as glutathione, methionine, metallothionein and protein. In the case of carboplatin, which has a more stable bidentate cyclobutanedicarboxylate ligand, the aquation reaction is much slower. This reduces drug potency, which thereby requires a greater dose for an equivalent antitumour effect. Indeed, since the final reactive species arising from cisplatin and carboplatin are identical, the slower rate of aquation may be the underlying basis for the reduced renal toxicity and peripheral neuropathy of carboplatin. This has led to the concept that high peak plasma concentrations of cisplatin are cytotoxic to both normal and tumour cells, whereas low sustained levels are equally effective against tumour cells but less toxic to normal cells. Support for the concept has come from clinical studies, which demonstrate the potential for an increase in the therapeutic index when cisplatin is given as a slow continuous infusion over several days (Salem *et al*., 1978, 1984).

Although activation is essential for activity, a substantially rapid generation of the reactive species is in general not conducive to antitumour effects. This may be particularly relevant in understanding why the highly reactive gold- or palladium-based agents are ineffective as antitumour agents. It is likely that these compounds are rapidly inactivated during nonspecific random interactions with plasma proteins and other components, and are therefore unable to reach the tumour site in sufficient concentrations to have any effect. Similarly, the clinical failure of the platinum(IV) agent ormaplatin could be ascribed to its rapid reduction to the platinum(II) form in the plasma and immediate inactivation of the transformed species through irreversible, noncytotoxic interactions with macromolecules such as plasma proteins (Siddik *et al*., 1999).

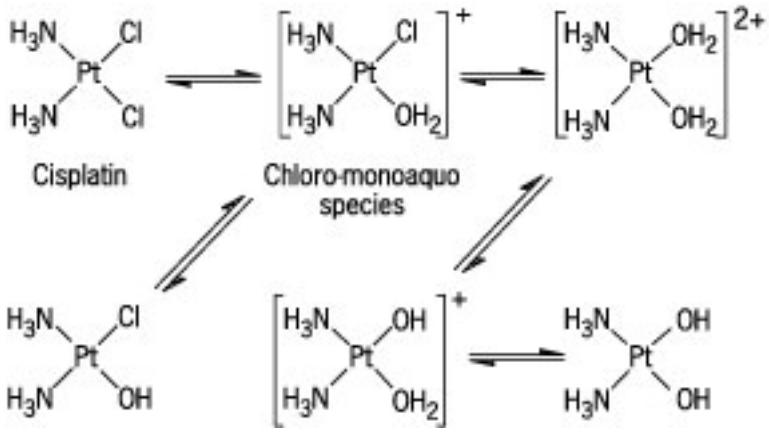

Figure 14 Conversion of cisplatin to positively charged reactive species via reversible aquation reactions.

DNA as a Target of Platinum Drugs

Studies conducted by several investigators, including Roberts and Pera (1983), leave little doubt that DNA is the primary target of cisplatin and other platinum agents. However, very little is known regarding the chemical form of cisplatin that reaches the nucleus. It is likely that the neutral uncharged species is the form that traverses the nuclear membrane. Although cisplatin enters cells through a predominantly nonsaturable passive diffusion process (Kelland, 2000), it is not known if a similar process also operates in nuclear drug uptake. Once inside the nucleus, the activated form of cisplatin interacts sequentially with nucleophilic sites on purine bases in DNA. First, as soon as the monoaquated species of cisplatin is formed, it reacts immediately with a DNA base (preferentially N7 of guanine) to form a monofunctional adduct. Such platinated adducts are considered inactive, as ascertained, for instance, from the inability of monofunctional adducts of cisplatin or transplatin to terminate RNA synthesis by bacterial RNA polymerases on DNA templates (Lemaire *et al*., 1991; Brabec and Leng, 1993). The remaining chloride ligand linked to platinum in the monoadduct is then hydrolysed, and the resulting aquated species interacts with a second nucleophilic site to form DNA and DNA–protein cross-links (Figure 15). Both 1,2- and 1,3-intrastrand DNA cross-links have been observed. The 1,2-interstrand DNA cross-links between opposite guanine bases are formed preferentially in 5′G–C3′ (G–C) sequences of both strands of linear DNA, but not in 5′C–G3′ (C–G) sequences as preferred by mitomycin C. The preference for G–C sequence for the formation of interstrand platinum cross-link is probably due to the relatively shorter distance between opposite guanines in G–C sequences (Malinge *et al*., 1999). Interestingly, interstrand cross-links are formed in both G–C and C–G sequences in supercoiled DNA, and this suggests that DNA topology can regulate interstrand platination reaction.

There is still uncertainty whether interstrand or intrastrand DNA cross-links are the cytotoxic lesions. Although interstrand cross-links can lead to biological effects, such as inhibition of transcriptional activity of prokaryotic and eukaryotic RNA polymerases on a damaged DNA template (Corda *et al*., 1991), the bulk of the evidence suggests that intrastrand adducts provide the strongest basis for the cytotoxic action of cisplatin, This is consistent with the knowledge that the relatively inactive transplatin cannot form intrastrand cross-links (Roberts and Friedlos, 1987). The substantial interest in intrastrand cross-links is also a result of biochemical analysis, which demonstrate that 1,2-intrastrand AG and GG cross-links account for about 85–90% of all DNA adducts (Kelland, 1993). In contrast, the 1,3-intrastrand GXG cross-links (where X is any nucleotide), interstrand GG cross-links and monofunctional adducts each make up about 2–6% of the platinum bound to DNA. The level of the AXG intrastrand adduct, on the other hand, is negligible. Although interstrand adduct levels are relatively low, they have also been correlated directly to cytotoxicity and, therefore, cannot be totally discounted (Roberts and Friedlos, 1987). The interstrand cross-links, on the other hand, are relatively unstable and convert to the more stable intrastrand form, with a half-life of about 29 h (Perez *et al*., 1997; Malinge *et al*., 1999). Similar levels of monoadducts and interstrand and intrastrand bi-adducts are also found for the analogue DACH-sulfatoplatinum(II) in an *in vitro* system (Jennerwein *et al*., 1989). Since cells resistant to cisplatin have only a low level of cross-resistance to this and other similar DACH-containing analogues (Eastman, 1987), it is reasonable to conclude that if the mechanism of action is at the DNA level, then the chemically specific adducts of cisplatin and DACH-based platinum agents (e.g. oxaliplatin) (Figure 16) must be a major determinant of the differential mode of action between the platinum drugs. Similarly, the chemical nature of adducts formed by cisplatin and carboplatin are identical, which is consistent with the knowledge that cisplatin-resistant tumours are cross-resistant to carboplatin.

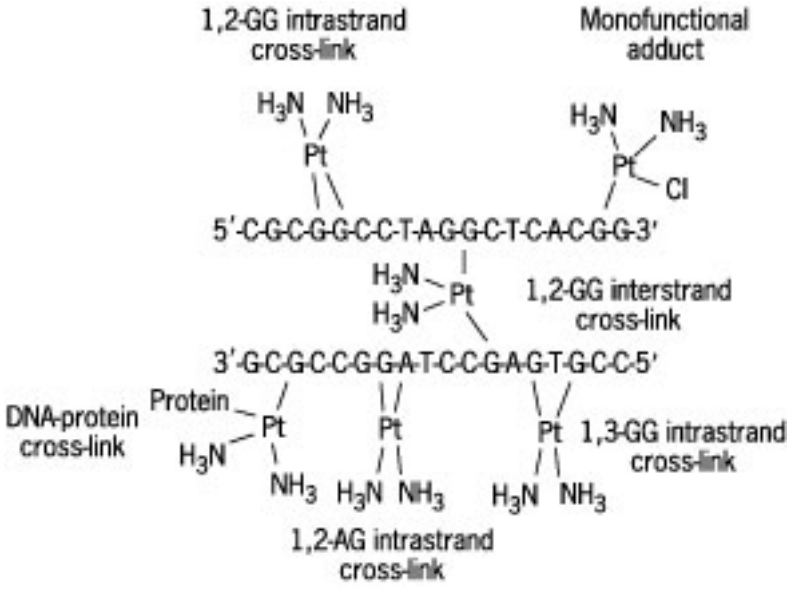

Figure 15 Types of DNA adducts and cross-links induced by cisplatin.

Compared with interstrand and intrastrand DNA cross-links, DNA–protein cross-links have been dismissed from playing a role in the cytotoxic process, partly on the basis of the finding that such lesions are formed extensively by the inactive agent transplatin (Zwelling *et al*., 1979).

Effect of Cross-Links on DNA Structure and Damage Recognition

It is widely understood that cross-linked adducts induced by cisplatin disrupt replication and transcriptional processes. Even just a few cross-links in the entire genome can be sufficient to inhibit DNA replication (Heiger-Bernays *et al*., 1990). Such biological effects, however, do not necessarily correlate directly with cytotoxic effects. Therefore, formation of cross-link lesions should merely be considered as the initial step in the complex process leading to cell death. Both interstrand and intrastrand cross-links induce local unwinding and bending in the DNA double helix. The AG, GG and GXG intrastrand bi-adducts of cisplatin unwind DNA by 13–23° and bend the double helix by 32–34° (Bellon *et al*., 1991). Interstrand cross-links, on the other hand, induce much greater effects: unwinding of 79° and greater, and bends of 45–47° have been reported (Malinge *et al*., 1999). Such physico-chemical characteristics may determine which signal transduction pathways are activated by interstrand and intrastrand adducts to induce cytotoxicity. Activation of these pathways probably occurs through special proteins with damage recognition properties that recognize the distinct distortions in the DNA and thereby affect cellular events, such as cell cycle arrest and apoptosis (a programmed form of cell death).

More than 20 different damage recognition proteins have been identified, and some specificity has been demonstrated by these proteins for DNA adducts of cisplatin and analogues. The mismatch repair (MMR) complex proteins, for instance, bind to cisplatin-induced DNA cross-links with much greater affinity than to those formed by oxaliplatin. The MMR appears to be essential for cisplatin sensitivity but is not involved in the mechanism of oxaliplatin-induced cytotoxicity (Chaney and Vaisman, 1999). Another important protein involved in recognition is the high mobility group 1 (HMG1) protein that recognizes cross-links of both cisplatin and oxaliplatin (Donahue *et al*., 1990), but the relative affinity again appears to be greater for those of cisplatin (Chaney and Vaisman, 1999). HMG1 binds to both intrastrand AG and GG adducts, but not to intrastrand GXG or monofunctional adducts. Interestingly, HMG1 also recognizes interstrand cross-links induced by cisplatin, but fails to interact with 1,1-cross-link of transplatin formed between guanine and the complementary cytosine residue (Kasparkova and Brabec, 1995). The TATA-binding protein (TBP), on the other hand, binds to adducts of both cisplatin and oxaliplatin with similar affinity (Chaney and Vaisman, 1999). In contrast, other damage recognition proteins, such as the Ku subunit of DNA-dependent protein kinase (DNA-PK), bind to DNA damage induced by either the active cisplatin or the inactive transplatin (Turchi *et al*., 1999). It is highly likely that each recognition protein initiates a specific molecular event, which may lead to cell death. Thus, differences between platinum analogues in their mode of action may be a result of differential recognition of individual distortions in DNA caused by drug-distinct bending and/or unwinding at the site of platination by the platinum analogue. The process, however, is probably more complex. For instance, intrastrand GG and GXG adducts induce similar bending and unwinding in DNA, but are differentially recognized by HMG1. It is very likely, therefore, that other factors contribute to the recognition process.

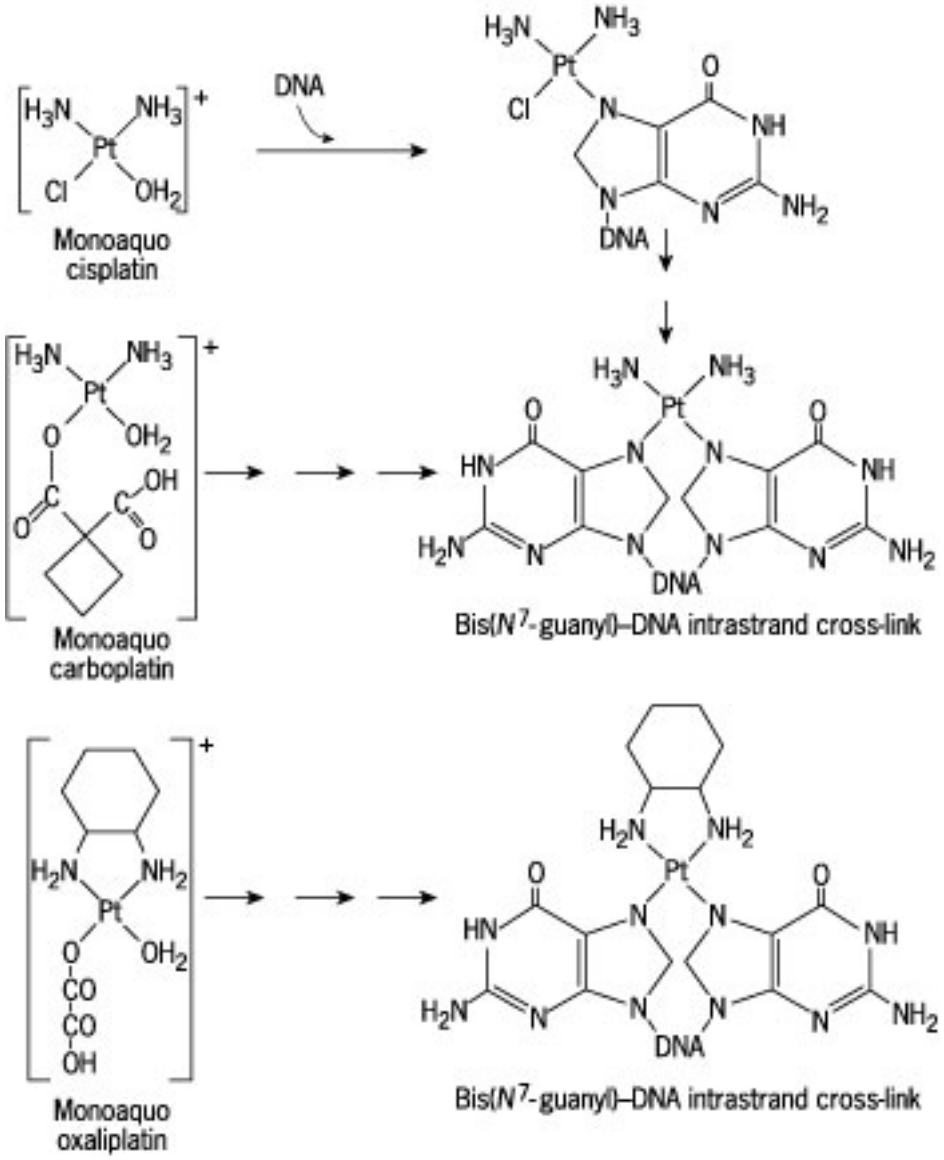

Figure 16 Cross-links between guanine bases induced by cisplatin, carboplatin and oxaliplatin. Note that cisplatin and carboplatin form an identical cross-link, whereas the cross-link of oxaliplatin is structurally very different by virtue of the bulky 1,2-diaminocyclohexane (DACH) group in the adduct.

The Role of the Tumour Suppressor p53

How the damage recognition proteins determine the fate of cells is not entirely clear. They have been implicated in shielding DNA adducts from repair that has the effect of increasing persistence of damage to facilitate cytotoxicity. This is consistent with the inverse relationship that an increase in nucleotide excision repair capacity of cells leads to a decrease in sensitivity of tumour cells to cisplatin. On the other hand, damage recognition proteins may play a role in activating signalling pathways, which affect a number of molecular events, including regulation of the tumour suppressor p53 protein (Kastan *et al*., 1991; Hainaut, 1995; Jayaraman *et al*., 1998). Normally, p53 is maintained intracellularly at very low levels or in an inactive state by its binding to the Mdm2 protein (Lakin and Jackson, 1999). When DNA is damaged by cisplatin, binding between Mdm2 and p53 is disrupted by phosphorylation of the tumour suppressor and results in p53 induction by virtue of a greater metabolic stability of the free p53 than of the p53–Mdm2 complex (Fritsche *et al*., 1993; Shieh *et al*., 1997; Lakin and Jackson, 1999). Once induced by the DNA damaging agents, p53 can transcriptionally activate DNA in a sequence-specific manner, eventually to give rise to other regulatory proteins such as $p21^{Waf1/Cip1}$ or Bax that can facilitate cell cycle arrest or cell death, respectively (Sionov and Haupt, 1999). However, the transcriptional activation by p53 is carefully orchestrated to provide a sequence of events that first results in cell cycle arrest through activation of cell cycle checkpoints to prevent not only DNA synthesis on a drug-damaged DNA template, but also segregation of damaged chromosomes during mitosis. If the cell cannot repair the damaged DNA, then apoptotic events are activated.

That p53 is a critical protein in protecting the genome and in preventing mutations in DNA from being passed on to daughter cells, comes from the realization that about 50% of all cancers have mutated p53, which has lost normal regulatory functions (Hollstein *et al*., 1991; Kastan *et al*., 1991; Hartwell and Kastan, 1994; Oltvai and Korsmeyer, 1994). In cancer chemotherapy, the intrinsic function of p53 to induce cell death and prevent damaged DNA to be propagated to normal daughter cells is exploited. It is not surprising, therefore, that the presence in tumours of mutant p53, compared with wild-type p53, reduces survival rates in patients treated with cisplatin for stage III/IV ovarian cancers (van der Zee *et al*., 1995). In such cases, combining cisplatin with gene therapy to restore wild-type p53 has become a viable therapeutic option. Presence of wild-type p53 in tumours, however, does not necessarily ensure greater sensitivity to cisplatin. Indeed, some tumour cell lines bearing wild-type p53 are highly resistant to cisplatin, and this has been attributed to a defective signalling pathway that fails to activate p53 following DNA damage as a result of cross-link formation. Interestingly, a DACH-containing analogue, (1*R*,2*R*)-DACH-(*trans*-diacetato)(dichloro)platinum(IV), is able to activate the dormant p53 and induce cytotoxicity (Hagopian *et al*., 1999; Siddik *et al*., 1999), which consolidates the belief that signalling transduced by DNA damage are different for cisplatin and such mechanistically distinct analogues. Although the activity of this DACH-based platinum(IV) compound was dependent on wild-type p53, it is known that cell cycle arrest and cell death can also occur in a p53-independent manner, which is not well understood (Michieli *et al*., 1994; Zhang *et al*., 1995; Segal-Bendirdjian *et al*., 1998; Haapajarvi *et al*., 1999). Furthermore, under certain conditions, inactivation of p53 can enhance cytotoxic sensitivity to cisplatin (Fan *et al*., 1995; Hawkins *et al*., 1996). These findings add credence to the understanding that cisplatin-induced cell death is a very complex process that will require greater knowledge to unravel the interplay between several signalling pathways that eventually determine whether a cell lives or dies. (See the chapter **Genomic Instability and DNA Repair**.)

Induction of Apoptosis

Members of the Bcl-2 family are also involved in the mechanism of action of cisplatin. Specific members are localized in the mitochondria and have either proapoptotic (Bax, Bak, Bid, Bim) or antiapoptotic (Bcl-2, Bcl-X_L, Bcl-W) functions (Farrow and Brown, 1996; Hanahan and Weinberg, 2000). These proteins form either homodimers (such as Bcl-2/Bcl-2) or heterodimers (e.g. Bcl-2/Bax) depending on the levels present of each component. Only an excess level of homodimers can either inhibit (e.g. Bcl-2/Bcl-2) or induce (e.g. Bax/Bax) apoptosis. Although there is no information available to indicate whether cisplatin can directly modulate levels of the antiapoptotic protein, there is evidence of a significant drug-mediated effect on Bax levels through transactivation of the *bax*. gene by wild-type p53. Thus, an increase in the Bax to Bcl-2 ratio by cisplatin-induced p53 has been reported to activate the apoptotic process (Eliopoulos *et al*., 1995). However, caution needs to be exercised in extrapolating experimental results to the clinic. For instance, the demonstration that experimental overexpression of *bcl-2* in tumours leads to the expected cisplatin resistance (Strasser *et al*., 1994; Herod *et al*., 1996; Miyake *et al*., 1999) is in sharp contrast to a clinical study, which reported that cisplatin surprisingly improved survival of patients with ovarian cancer that demonstrated increased *bcl-2* gene expression (Herod *et al*., 1996). Our present understanding indicates that proapoptotic homodimers affect cisplatin-induced apoptosis

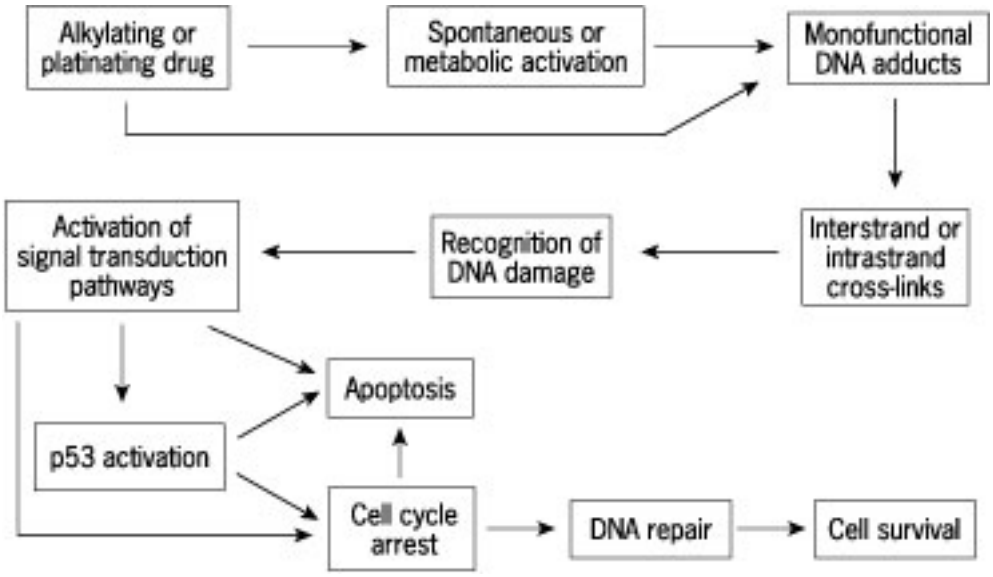

Figure 17 A general scheme for DNA-interactive agents that proposes critical events leading to DNA damage and subsequent cell survival or apoptotic form of cell death.

by first stimulating the mitochondria to release cytochrome *c*, which in turn activates a series of proteases that includes caspase-1, -3 and -9 (Kondo *et al*., 1995; Henkels and Turchi, 1999; Gebauer *et al*., 2000; Hanahan and Weinberg, 2000). These proteases appear to be the final effectors of drug-mediated apoptotic cell death. (See the chapter **Apoptosis**.)

CONCLUSION

From the above discussions, we can formulate a general understanding for the mechanism of action of alkylating and platinating agents. Although much of the information has been derived from studies with platinum-based drugs, the general principles most likely apply to alkylating agents also. Once these antitumour agents are activated, they damage DNA by forming monofunctional adducts and interstrand and intrastrand cross-links, which cause DNA to unwind and/or bend. Such distortions are then recognized by specialized DNA damage recognition proteins, and a cascade of events is activated that leads to p53-dependent or -independent cell cycle arrest to allow time for DNA repair. If repair is incomplete, p53-dependent or -independent programmed cell death (apoptosis) is initiated to complete an orderly process of cell destruction. Figure 17 summarizes this general sequence of events, and suggests that any factor interfering with this scheme, such as reduced adduct formation (e.g. drug inactivation by glutathione) or persistence (e.g. enhanced repair), reduced recognition of damage (e.g. mutation in mismatch repair complex), aberrant signal transduction pathways (e.g. mutation in *p53*), and reduced apoptotic activity (e.g. *p53* mutation or increased *bcl-2* overexpression), will lead to resistance to alkylating and platinating drugs.

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REFERENCES

Adair, F. E. and Bagg, H. J. (1931). Experimental and clinical studies on the treatment of cancer by dichloroethyl sulfide (mustard gas). *Annals of Surgery*, **93**, 190–199.

Ali-Osman, F., *et al*. (1985). Chemical structure of carbamoylating groups and their relationship to bone marrow toxicity and antiglioma activity of bifunctional alkylating and carbamoylating nitrosoureas. *Cancer Research*, **45**, 4185–4191.

Andrievesky, G. V., *et al*. (1991). Direct observation of the alkylation products of deoxyguanosine and DNA by fast atom bombardment mass spectrometry. *Biological Mass Spectrometry*, **20**, 665–668.

Bedford, P. and Fox, B. W. (1983). DNA–DNA interstrand crosslinking by dimethylsulphonic acid esters. Correlation with cytotoxicity and antitumour activity in the Yoshida lymphosarcoma model and relationship to chain length. *Biochemical Pharmacology*, **32**, 2297–2301.

Bellon, S. F., *et al*. (1991). DNA unwinding produced by site-specific intrastrand cross-links of the antitumor drug *cis*-diamminedichloroplatinum(II). *Biochemistry*, **30**, 8026–8035.

Brabec, V. and Leng, M. (1993). DNA interstrand cross-links of *trans*-diamminedichloroplatinum(II) are preferentially formed between guanine and complementary cytosine residues. *Proceedings of the National Academy of Sciences of the USA*, **90**, 5345–5349.

Chaney, S. G. and Vaisman, A. (1999). Specificity of platinum–DNA adduct repair. *Journal of Inorganic Biochemistry*, **77**, 71–81.

Colvin, M. (1982). The comparative pharmacology of cyclophosphamide and ifosfamide. *Seminars in Oncology*, **9**, 2–7.

Colvin, M. E., *et al*. (1999). Chemical factors in the action of phosphoramidic mustard alkylating anticancer drugs: roles for computational chemistry. *Current Pharmaceutical Design*, **5**, 645–663.

Corda, Y., *et al*. (1991). Transcription by eucaryotic and procaryotic RNA polymerases of DNA modified at a d(GG) or a d(AG) site by the antitumor drug *cis*-diamminedichloroplatinum(II). *Biochemistry*, **30**, 222–230.

Cox, P. J. (1979). Cyclophosphamide cystitis – identification of acrolein as the causative agent. *Biochemical Pharmacology*, **28**, 2045–2049.

D'Incalci, M., *et al*. (1992). Sequence and gene-specific drugs. In: Workman, P. (ed.), *New Approaches in Cancer Pharmacology: Drug Design and Development*. 5–11. (Springer-Verlag, Berlin).

Donahue, B. A., *et al*. (1990). Characterization of a DNA damage-recognition protein from mammalian cells that binds specifically to intrastrand d(GpG) and d(ApG) DNA adducts of the anticancer drug cisplatin. *Biochemistry*, **29**, 5872–5880.

Eastman, A. (1987). Glutathione-mediated activation of anticancer platinum(IV) complexes. *Biochemical Pharmacology*, **36**, 4177–4178.

Egorin, M. J. and Snyder, S. W. (1990). Characterization of nonexchangeable radioactivity in L1210 cells incubated with [^{14}C]thiotepa: labeling of phosphatidylethanolamine. *Cancer Research*, **50**, 4044–4049.

Eisenbrand, G., *et al*. (1986). DNA adducts and DNA damage by antineoplastic and carcinogenic *N*-nitroso compounds. *Journal of Cancer Research and Clinical Oncology*, **112**, 196–204.

Eisenhauer, E., *et al*. (1990). Carboplatin therapy for recurrent ovarian carcinoma: National Cancer Institute of Canada experience and a review of the literature. In: Bunn, P. *et al*., (eds), *Carboplatin: Current Perspectives and Future Directions*. 133–40. (W.B. Saunders Company, Philadelphia).

el Khateeb, M., *et al*. (1999). Reactions of cisplatin hydrolytes with methionine, cysteine, and plasma ultrafiltrate studied by a combination of HPLC and NMR techniques. *Journal of Inorganic Biochemistry*, **77**, 13–21.

Eliopoulos, A. G., *et al*. (1995). The control of apoptosis and drug resistance in ovarian cancer: influence of p53 and Bcl-2. *Oncogene*, **11**, 1217–1228.

Faivre, S., *et al*. (1999). Oxaliplatin and paclitaxel combination in patients with platinum-pretreated ovarian carcinoma: an investigator-originated compassionate-use experience. *Annals of Oncology*, **10**, 1125–1128.

Fan, S., *et al*. (1995). Disruption of p53 function sensitizes breast cancer MCF-7 cells to cisplatin and pentoxifylline. *Cancer Research*, **55**, 1649–1654.

Farrow, S. N. and Brown, R. (1996). New members of the Bcl-2 family and their protein partners. *Current Opinions in Genetic Development*, **6**, 45–49.

Fritsche, M., *et al*. (1993). Induction of nuclear accumulation of the tumor-suppressor protein p53 by DNA-damaging agents. *Oncogene*, **8**, 307–318.

Garcia, S.T., *et al*. (1988). Correlation between the cytotoxicity of melphalan and DNA crosslinks as detected by the ethidium bromide

fluorescence assay in the F1 variant of B16 melanoma cells. *Biochemical Pharmacology*, **37**, 3189–3192.

Gebauer, G., *et al*. (2000). Cisplatin-resistance involves the defective processing of MEKK1 in human ovarian adenocarcinoma 2008/C13 cells. *International Journal of Oncology*, **16**, 321–325.

Gilman, A. and Phillips, F. S. (1946). The biological actions and therapeutic applications of β-chloroethyl amines and sulfides. *Science*, **103**, 409–436.

Gore, M., *et al*. (1989). Cisplatin/carboplatin cross-resistance in ovarian cancer. *British Journal of Cancer*, **60**, 767–769.

Haapajarvi, T., *et al*. (1999). Human melanoma cell line UV responses show independency of p53 function. *Cell Growth of Differences*, **10**, 163–171.

Hagopian, G. S., *et al*. (1999). Expression of p53 in cisplatin-resistant ovarian cancer cell lines: modulation with the novel platinum analogue (1R, 2R-diaminocyclohexane)(trans-diacetato)(dichloro)-platinum(IV). *Clinical Cancer Research*, **5**, 655–663.

Hainaut, P. (1995). The tumor suppressor protein p53: a receptor to genotoxic stress that controls cell growth and survival. *Current Opinion in Oncology*, **7**, 76–82.

Hanahan, D. and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell*, **100**, 57–70.

Hartwell, L. H. and Kastan, M. B. (1994). Cell cycle control and cancer. *Science*, **266**, 1821–1828.

Hawkins, D. S., *et al*. (1996). Inactivation of p53 enhances sensitivity to multiple chemotherapeutic agents. *Cancer Research*, **56**, 892–898.

Heiger-Bernays, W. J., *et al*. (1990). Effect of the antitumor drug *cis*-diamminedichloroplatinum(II) and related platinum complexes on eukaryotic DNA replication. *Biochemistry*, **29**, 8461–8466.

Henkels, K. M. and Turchi, J. J. (1999). Cisplatin-induced apoptosis proceeds by caspase-3-dependent and -independent pathways in cisplatin-resistant and -sensitive human ovarian cancer cell lines. *Cancer Research*, **59**, 3077–3083.

Herod, J. J., *et al*. (1996). The prognostic significance of Bcl-2 and p53 expression in ovarian carcinoma. *Cancer Research*, **56**, 2178–2184.

Hollstein, M., *et al*. (1991). p53 mutations in human cancers. *Science*, **253**, 49–53.

Jayaraman, L., *et al*. (1998). High mobility group protein-1 (HMG-1) is a unique activator of p53. *Genes and Development*, **12**, 462–472.

Jennerwein, M. M., *et al*. (1989). Characterization of adducts produced in DNA by isomeric 1,2-diaminocyclohexaneplatinum(II) complexes. *Chemical–Biological Interactions*, **70**, 39–49.

Kasparkova, J. and Brabec, V. (1995). Recognition of DNA interstrand cross-links of *cis*-diamminedichloroplatinum(II) and its *trans* isomer by DNA-binding proteins. *Biochemistry*, **34**, 12379–12387.

Kastan, M. B., *et al*. (1991). Participation of p53 protein in the cellular response to DNA damage. *Cancer Research*, **51**, 6304–6311.

Kelland, L.R. (1993). New platinum antitumor complexes. *Critical Reviews of Oncology and Hematology*, **15**, 191–219.

Kelland, L. R. (2000). Preclinical perspectives on platinum resistance. *Drugs*, **59** (Suppl. 4), 1–8.

Kelland, L.R., *et al*. (1999). Mini-review: discovery and development of platinum complexes designed to circumvent cisplatin resistance. *Journal of Inorganic Biochemistry*, **77**, 111–115.

Kondo, S., *et al*. (1995). MDM2 protein confers the resistance of a human glioblastoma cell line to cisplatin-induced apoptosis. *Oncogenetics*, **10**, 2001–2006.

Lakin, N. D. and Jackson, S. P. (1999). Regulation of p53 in response to DNA damage. *Oncogene*, **18**, 7644–7655.

Lawley, P. D. and Brookes, P. (1965). Molecular mechanism of the cytotoxic action of difunctional alkylating agents and of resistance to this action. *Nature*, **206**, 480–483.

Lemaire, M. A., *et al*. (1991). Interstrand cross-links are preferentially formed at the d(GC) sites in the reaction between *cis*-diamminedichloroplatinum(II) and DNA. *Proceedings of the National Academy of Sciences of the USA*, **88**, 1982–1985.

Lemoine, A., *et al*. (1991). Metabolism of the chloroethylnitrosoureas. *Xenobiotica*, **21**, 775–791.

Ludlum, D. B. (1997). The chloroethylnitrosoureas: sensitivity and resistance to cancer chemotherapy at the molecular level. *Cancer Investigations*, **15**, 588–598.

Maanen, M. J., *et al*. (2000). Chemistry, pharmacology and pharmacokinetics of *N,N′,N′′*-triethylenethiophosphoramide (ThioTEPA). *Cancer Treatment Reviews*, **26**, 257–268.

Malinge, J. M., *et al*. (1999). Interstrand cross-links of cisplatin induce striking distortions in DNA. *Journal of Inorganic Biochemistry*, **77**, 23–29.

Michieli, P., *et al*. (1994). Induction of WAF1/CIP1 by a p53-independent pathway. *Cancer Research*, **54**, 3391–3395.

Miyake, H., *et al*. (1999). Synergistic enhancement of resistance to cisplatin in human bladder cancer cells by overexpression of mutant-type p53 and Bcl-2. *Journal of Urology*, **162**, 2176–2181.

Musser, S. M., *et al*. (1992). Alkylation of DNA with aziridine produced during the hydrolysis of N,N′,N-triethylenethiophosphoramide. *Chemical Research Toxicology*, **5**, 95–99.

Oltvai, Z. N. and Korsmeyer, S. J. (1994). Checkpoints of dueling dimers foil death wishes. *Cell*, **79**, 189–192.

Perez, C., *et al*. (1997). Rearrangement of interstrand cross-links into intrastrand cross-links in cis-diamminedichloroplatinum(II)-modified DNA. *Nucleic Acids Research*, **25**, 896–903.

Perez, J. M., *et al*. (1999). Apoptosis induction and inhibition of H-ras overexpression by novel trans-[$PtCl_2$(isopropylamine)-(amine′)] complexes. *Journal of Inorganic Biochemistry*, **77**, 37–42.

Pratt, W. B., *et al*. (1994). *The Anticancer Drugs* (Oxford University Press, New York).

Prestayko, A.W., *et al*. (1979). Cisplatin (*cis*-diamminedichloroplatinum II). *Cancer Treatment Reviews*, **6**, 17–39.

Reed, D. J. (1987). 2-Chloroethylnitrosoureas. In: Powis, G. and Prough, R. A. (eds), *Metabolism and Action of Anti-Cancer Drugs*. 1–28. (Taylor and Francis, London).

Roberts, J. J. and Friedlos, F. (1987). Quantitative estimation of cisplatin-induced DNA interstrand cross-links and their repair in mammalian cells: relationship to toxicity. *Pharmacological Therapeutics*, **34**, 215–246.

Roberts, J. J. and Pera, M. F., Jr. (1983). DNA as a target for anticancer coordination compounds. In: Lippard, S. J. (ed.), *Platinum, Gold, and Other Metal Chemotherapeutic Agents: Chemistry and Biochemistry*. 3–25. (American Chemical Society, Washington, DC).

Roberts, J. J., *et al*. (1968). The mechanism of the cytotoxic action of alkylating agents on mammalian cells. In: Campbell, P. M. (ed.), *The Interaction of Drugs and Subcellular Components in Animal Cells*. 5–27. (Churchill, London).

Rosenberg, B. (1980). Cisplatin: its history and possible mechanisms of action. In: Prestayko, A. W., *et al*. (eds), *Cisplatin: Current Status and New Developments*. 9–20. (Academic Press, London).

Salem, P., *et al*. (1978). Clinical phase I–II study of cis-dichlorodiammineplatinum(II) given by continuous iv infusion. *Cancer Treatment Representatives*, **62**, 1553–1555.

Salem, P., *et al*. (1984). *cis*-Diamminedichloroplatinum(II) by 5-day continuous infusion. A new dose schedule with minimal toxicity. *Cancer*, **53**, 837–840.

Segal-Bendirdjian, E., *et al*. (1998). Alteration in p53 pathway and defect in apoptosis contribute independently to cisplatin-resistance. *Cell Deaths of Differences*, **5**, 390–400.

Shieh, S. Y., *et al*. (1997). DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. *Cell*, **91**, 325–334.

Siddik, Z. H. and Newman, R. A. (1994). Metabolism of new anti-cancer agents. In: Powis, G. (ed.), *Anticancer Drugs: Antimetabolite Metabolism and Natural Anticancer Agents*. 437–480. (Pergamon Press, Oxford).

Siddik, Z. H., *et al*. (1999). Role of p53 in the ability of 1,2-diaminocyclohexane-diacetato-dichloro-Pt(IV) to circumvent cisplatin resistance. *Journal of Inorganic Biochemistry*, **77**, 65–70.

Sionov, R. V. and Haupt, Y. (1999). The cellular response to p53: the decision between life and death. *Oncogenetics*, **18**, 6145–6157.

Sladek, N. E. (1987). Oxazaphosphorines. In: Powis, G. and Prough, R. A. (eds), *Metabolism and Action of Anti-cancer Drugs*. 48–90. (Taylor and Francis, London).

Strasser, A., *et al*. (1994). DNA damage can induce apoptosis in proliferating lymphoid cells via p53-independent mechanisms inhibitable by Bcl-2. *Cell*, **79**, 329–339.

Tanimura, H., *et al*. (1995). Identification of non-cross resistant platinum compounds among agents submitted to the National Cancer Institute's (NCI's) anticancer drug screen using the DISCOVERY computer program (Meeting abstract). *Proceedings of the Annual Meeting of the American Association for Cancer Research*, **36**, A1816.

Tong, W. P. and Ludlum, D. B. (1980). Crosslinking of DNA by busulfan. Formation of diguanyl derivatives. *Biochimica et Biophysica Acta*, **608**, 174–181.

Turchi, J. J., *et al*. (1999). Interactions of mammalian proteins with cisplatin-damaged DNA. *Journal of Inorganic Biochemistry*, **77**, 83–87.

van der Zee, A. G., *et al*. (1995). Value of P-glycoprotein, glutathione S-transferase pi, c-erbB-2, and p53 as prognostic factors in ovarian carcinomas. *Journal of Clinical Oncology*, **13**, 70–78.

Vistica, D. T. (1979). Cytotoxicity as an indicator for transport mechanism: evidence that melphalan is transported by two leucine-preferring carrier systems in the L1210 murine leukemia cell. *Biochimica et Biophysica Acta*, **550**, 309– 317.

Yeager, A. M., *et al*. (1986). Autologous bone marrow transplantation in patients with acute nonlymphocytic leukemia, using ex vivo marrow treatment with 4-hydroperoxycyclophosphamide. *New England Journal of Medicine*, **315**, 141–147.

Zhang, W., *et al*. (1995). p53-independent induction of WAF1/CIP1 in human leukemia cells is correlated with growth arrest accompanying monocyte/macrophage differentiation. *Cancer Research*, **55**, 668–674.

Zwelling, L. A., *et al*. (1979). DNA-protein and DNA interstrand cross-linking by *cis*- and *trans*-platinum(II) diamminedichloride in L1210 mouse leukemia cells and relation to cytotoxicity. *Cancer Research*, **39**, 365–369.

FURTHER READING

Chaney, S. G. and Vaisman, A. (1999). Specificity of platinum-DNA adduct repair. *Journal of Inorganic Biochemistry*, **77**, 71–81.

Colvin, M. E., *et al*. (1999). Chemical factors in the action of phosphoramidic mustard alkylating anticancer drugs: roles for computational chemistry. *Current Pharmaceutical Design*, **5**, 645–663.

Hanahan, D. and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell*, **100**, 57–70.

Hartwell, L. H. and Kastan, M. B. (1994). Cell cycle control and cancer. *Science*, **266**, 1821–1828.

Lakin, N. D. and Jackson, S. P. (1999). Regulation of p53 in response to DNA damage. *Oncogene*, **18**, 7644–7655.

Ludlum, D. B. (1997). The chloroethylnitrosoureas: sensitivity and resistance to cancer chemotherapy at the molecular level. *Cancer Investigations*, **15**, 588–598.

Maanen, M. J., *et al*. (2000). Chemistry, pharmacology and pharmacokinetics of *N,N′,N′′*-triethylenethiophosphoramide (ThioTEPA). *Cancer Treatment Reviews*, **26**, 257–268.

Malinge, J. M., *et al*. (1999). Interstrand cross-links of cisplatin induce striking distortions in DNA. *Journal of Inorganic Biochemistry*, **77**, 23–29.

Reed, D. J. (1987). 2-Chloroethylnitrosoureas. In: Powis, G. and Prough, R. A. (eds), *Metabolism and Action of Anti-Cancer Drugs*. 1–28. (Taylor and Francis, London).

Sionov, R. V. and Haupt, Y. (1999). The cellular response to p53: the decision between life and death. *Oncogene*, **18**, 6145–6157.